



## **Wet STEM in SEM for Morphological Characterization of Novel Bacterial Species: *Vibrio galathea* and *Photobacterium galathea***

**Mateiu, Ramona Valentina; Giubergia, Sonia; Machado, Henrique; Gram, Lone; Wagner, Jakob Birkedal**

*Publication date:*  
2016

*Document Version*  
Peer reviewed version

[Link back to DTU Orbit](#)

*Citation (APA):*

Mateiu, R. V., Giubergia, S., Machado, H., Gram, L., & Wagner, J. B. (2016). *Wet STEM in SEM for Morphological Characterization of Novel Bacterial Species: Vibrio galathea and Photobacterium galathea*. Abstract from 16th European Microscopy Congress, Lyon, France.

---

### **General rights**

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

## Wet STEM in SEM for Morphological Characterization of Novel Bacterial Species: *Vibrio galathea* and *Photobacterium galathea*

Ramona Valentina Mateiu<sup>1</sup>, Sonia Giubergia<sup>2,3</sup>, Henrique Machado<sup>2,3</sup>, Lone Gram<sup>2</sup> and Jakob Birkedal Wagner<sup>1</sup>

<sup>1</sup> Center for Electron Nanoscopy, Danchip, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark

<sup>2</sup> Department of Systems Biology, Technical University of Denmark, Matematiktorvet, bldg 301, DK-2800 Kgs. Lyngby, Denmark

<sup>3</sup> Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kogle Allé 6, DK-2970 Hørsholm, Denmark 3

Scanning electron microscope (SEM) is increasingly used for morphology characterization in various research fields. The technique is appealing due to the fact that it has a fairly large field of view ( $\mu\text{m}$  to  $\text{mm}$ ) with  $\text{nm}$  lateral resolution, which makes it suitable for characterization of micrometer scale objects, which have  $\text{nm}$  scale features, such as bacteria. The major limiting factors for SEM as a visualization tool of bacteria are the sample preparation, which is prone to artefacts, and the sample interaction volume, which limits the lateral resolution. Here, we present an approach for morphology characterization, which involves minimal sample preparation and maximized lateral resolution: the use of environmental SEM (E-SEM) with a scanning transmission electron detector (STEM); wet STEM. We use wet STEM for morphological characterization (identification of cell shape, size and appendices) of two bacteria isolated from a mussel collected in the Solomon Sea (Solomon Islands) during the Danish research expedition Galathea 3: *Vibrio galathea*<sup>1</sup> and *Photobacterium galathea*<sup>2</sup>.

The bacteria were grown in marine broth in flask cultures. After 24h growth at 25°C the marine broth was washed with miliQ water and a 0.5  $\mu\text{L}$  droplet placed on a plasma treated carbon film 200 mesh copper grid. The sample was negatively stained with 2 % uranyl acetate aqueous solution and imaged in an FEI Quanta 200 FEG E-SEM with an electron beam with spot 3 accelerated to 15 keV. During electron microscopy the bacterial cells were kept fully hydrated by using water as auxiliary gas and continuously condensing water on the sample. The bacteria were imaged using the transmitted electrons and a STEM detector (a 2 quad, diode back scattered electron detector mounted below the sample).

The wet STEM micrographs revealed that the *Vibrio galathea*, has *Vibrio* monotrichous cells,  $1.482 \pm 0.365 \mu\text{m}$  long,  $0.743 \pm 0.181 \mu\text{m}$  width and  $3.625 \pm 0.639 \mu\text{m}$  long flagellum (Figure 1), and the *Photobacterium galathea* has *Bacillus* monotrichous cells,  $2.261 \pm 0.404 \mu\text{m}$  long,  $0.985 \pm 0.128 \mu\text{m}$  wide and  $4.306 \pm 1.094 \mu\text{m}$  long flagellum (Figure 2). Hence, we show that the wet STEM in SEM analysis is a quick and efficient technique that can be easily used for identification of cell shape, appendices and size measurements of bacteria.

### References

1. *Vibrio galathea* sp. nov., a member of the family Vibrionaceae isolated from a mussel, Sonia Giubergia, Henrique Machado, Ramona Valentina Mateiu and Lone Gram, International Journal of Systematic and Evolutionary Microbiology (2016), 66, 347–352.
2. *Photobacterium galathea* sp. nov., a bioactive bacterium isolated from a mussel in the Solomon Sea, Henrique Machado, Sonia Giubergia, Ramona Valentina Mateiu and Lone Gram, International Journal of Systematic and Evolutionary Microbiology (2015), 65, 4503–4507.

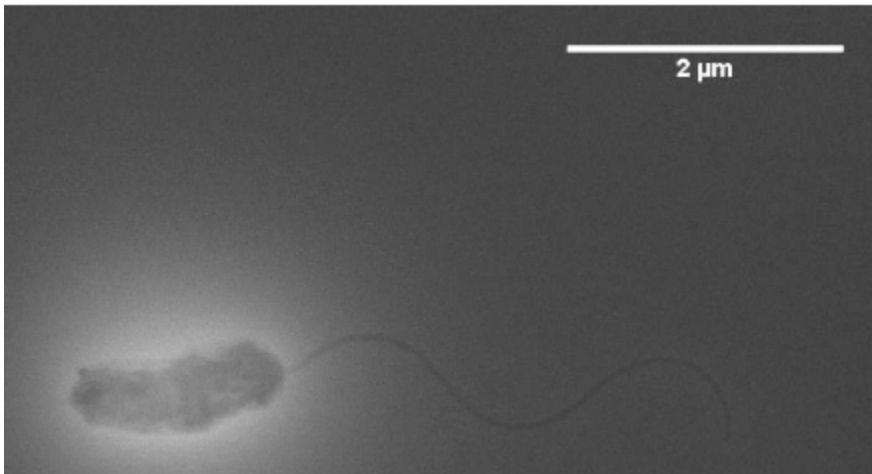


Figure 1. Wet STEM micrograph of a *Vibrio galathea* cell.

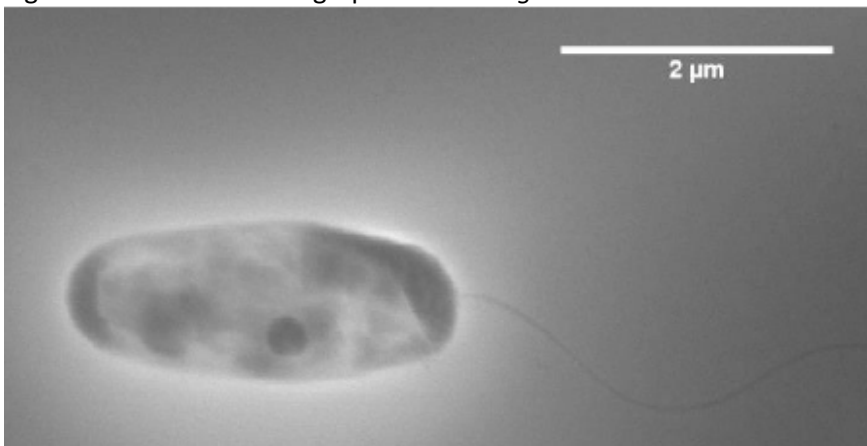


Figure 2. Wet STEM micrograph of a *Photobacterium galathea* cell.